J. Natn. Sci. Coun. Sri Lanka 1998 **26** *(2):133-139*

STRUCTURE-ANTIBACTERIAL ACTIVITY RELATIONSHIP OF SOME AROMATIC ACIDS

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(Received: 02 December 1997; accepted: 05 June 1998)

Abstract: Nine aromatic acids were tested for their antibacterial effect against *Staphylococcus aureus*. 3-phenylpropanoic acid was identified as the most active of the acids chosen for this bioassay. In general a 3,4-methylenedioxy substituent on the phenyl group reduces the activity against *Staphylococcus aureus*.

Keywords: Antibacterial action, aromatic acids, *Staphylococcus aureus*.

INTRODUCTION

Benzoic acid, the simplest aromatic acid is well known for its antimicrobial activity[1] and is widely used as a food preservative. Cinnamic acid derivatives have also been studied for possible antibacterial and antifungal activity. Ring substituted cinnamic acids[2] (-methoxy, -amino, -chloro, -bromo, -hydroxy) have been tested against different bacteria and fungi and the halogenated derivatives have shown the highest activity against the tested organisms. *trans*-Ethyl p-methoxycinnamate[3] is also known to inhibit *in vitro*, the growth of several fungi at 10-50 μg/l. Geometry around the double bond is a key factor for the antifungal property of this compound, since the reduction of the double bond or conversion into the *cis*-isomer by UV exposure resulted in total loss of anti-fungal activity.

The effect of piperine[4] (the pyrrolidine amide of piperic acid) on the growth of microorganisms has been studied and the minimum inhibitory concentration required was greater than 100 μg/ml against all the tested organisms. Piperine, the principal alkaloid in pepper has been used in curing of meat by mankind for generations. The action of pepper on meat is scientifically explained as piperine inhibits the growth of fungi and bacteria. A recent study[5] performed in our laboratory indicates that the piperic acid itself shows anti-bacterial activity against *Staphylococcus aureus*.

METHODS AND MATERIALS

The following two series of ten aromatic acids were chosen to conduct this study based on their structural relationship to benzoic, cinnamic and piperic acid which enabled us to find out the variation of antibacterial activity upon changing the length and saturation of the carbon chain attached to the phenyl ring.

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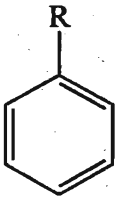
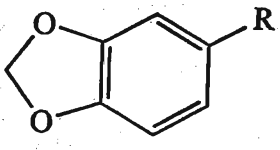
Materials:

Series 1:



Compound	R	IUPAC/Common name
1	-COOH	Benzoic acid
2	-CH=CH-COOH	E-3-Phenylpropenoic acid/Cinnamic acid
3	$-CH_2CH_2COOH$	3-Phenylpropanoic acid/Hydrocinnamic acid
4	-CH=CH-CH=CH-COOH	5-Phenylpenta-2,4-dienoic acid
5	$-CH_2CH_2CH_2CH_2COOH$	5-Phenylpentanoic acid/5-Penylvaleric acid

Series 2:



Compound	R	IUPAC/Common name
6	-COOH	3,4-Methylenedioxybenzoic acid/Piperonylic acid
7	-CH=CH-COOH	E-3-(3,4-Methylenedioxyphenyl)propenoic acid
8	$-CH_2CH_2COOH$	3-(3,4-Methylenedioxyphenyl)propanoic acid
9	-CH=CH-CH=CH-COOH	E-5-(3,4-Methylenedioxyphenyl)penta-2,4-dienoic acid/ Piperic acid
10	$-CH_2CH_2CH_2CH_2COOH$	5-(3,4-Methylenedioxyphenyl)pentanoic acid
*11	*-CHO*	*Piperonal*

Benzoic acid (**1**), cinnamic acid (**2**) and piperic acid (**9**) were of BDH manufacture. Compound **3** was obtained from **2** and compound **5** from **4** by catalytic hydrogenation. Similar procedures were adopted in the preparation of compounds **8** from **7** and **10** from **9**. Compound **4** was obtained from the reaction between cinnamaldehyde and malonic acid and compound **7** was synthesized by a similar reaction of malonic acid with piperonal (***11***). Compound **6** was obtained from the oxidation of piperonal.

Methods

Catalytic hydrogenation:

The acid (3 mmol) was dissolved in methanol (10 ml) and 10% Pd on activated charcoal (100 mg) was added to the reaction. The reaction flask was connected to a balloon filled with H_2 through an adapter and the solution was stirred vigorously for 4 h. The mixture was filtered to remove the catalyst and the solvent was concentrated to obtain the product.

Condensation of malonic acid with piperonal:

Piperonal (0.5g, 3.3 mmol) and malonic acid (0.75g, 7.2 mmol) were dissolved in pyridine (1ml) and a few drops of piperidine added. The mixture was refluxed for 1 h, cooled to room temperature and poured into 5% HCl (10ml) while stirring. The precipitated product was filtered and washed with cold water. Recrystallization from methanol gave **7**.

Similar reaction of cinnamaldehyde with malonic acid gave compound **4** which was recrystallized from water.

Oxidation of piperonal:

A solution of $KMnO_4$ (1.5g) in water (30 ml) was added to piperonal (1g, 6.6 mmol) in wate r (25 ml) in a 100 ml flask while stirring on a steam bath at 70-80°C over 15 min. Stirring and heating was continued for 1/2h and the solution was made alkaline by adding 10% KOH. The mixture was filtered while hot and the MnO_2 residue was washed with hot water. The filtrate was allowed to cool and acidified with conc. HCl until no further precipitate was formed. Recrystallization from ethanol gave **6**.

Compounds were identified by NMR spectroscopy of $CDCl_3$ solutions on a 200 MHz Bruker spectrometer using tetramethylsilane (TMS) as the internal reference.

Agar plate assay:

A drop of a 24 h old culture of the test bacterium *Staphylococcus aureus* in marmite peptone liquid medium was placed on a nutrient agar plate (20 ml nutrient agar in 9 cm petri dish) and spread over the surface using a glass spreader. Thereafter, the test solution (5ml-25ml volumes from 10,000 ppm solutions) was absorbed onto sterile filter paper discs of 6 mm diameter, and the discs placed on the innoculated agar plate. In the control, an equal volume of the solvent (ethyl acetate) was absorbed onto sterile filter paper disc. Each experiment was carried out in triplicate. The inoculated plates with the sterile filter paper discs were incubated for 48 h at 37°C and observed for inhibitory zones. The diameters of inhibitory zones were measured at the end of the incubation period.

RESULTS

Hydrocinnamic acid **3** was obtained as a yellow oil from cinnamic acid in 92% yield.
^{1}H NMR δ 10.20 (s, broad), 7.33-7.18 (m, 5H, aromatic), 2.95 (t, 2H, *J*=7.7 Hz), 2.67 (t, 2H, *J*=7.7 Hz); ^{13}C NMR δ 179.4, 140.1, 128.5, 128.2, 126.3, 35.6, 30.5

5-Phenylpenta-2,4-dienoic acid **4** was obtained as an orange solid which recrystallized from water as yellow plates in 11% yield., m.p. 166-167°C (Lit[6]. 165-166°C) ^{1}H NMR δ 7.61-7.30 (m, 6H), 6.93 (m, 2H), 6.01 (d, 1H, *J*=15.2); ^{13}C NMR δ 171.8, 146.9, 141.6, 135.8, 129.3, 128.8, 127.3, 125.9, 120.1

5-Phenylpentanoic acid **5** was obtained as a yellow oil from acid **4** in 8% yield. ^{1}H NMR δ 8.20 (s, broad), 7.30-7.11 (m, 5H), 2.66 (m, 2H), 2.39 (m, 2H), 1.65 (m, 4H); ^{13}C NMR δ 180.0, 141.9, 128.3, 125.8, 35.5, 33.9, 30.7, 24.3.

Piperonylic acid **6** was obtained as white needles in 9% yield. m.p. 228°C (Lit[7]. 229 °C) ^{1}H NMR δ 7.72 (dd, 1H, *J*= 1.7 and 8.2 Hz), 7.51 (d, 1H, *J*=1.7 Hz), 6.87 (d, 1H, *J*=8.2 Hz), 6.08 (s, 2H); ^{13}C DEPT-45 δ 126.3, 109.9, 108.0, 101.9.

E-3-(3,4-Methylenedioxyphenyl)propenoic acid **7** was obtained as pale yellow needles in 69% yield. m.p. 258-260°C (Lit[6]. 242°C) ^{1}H NMR δ 8.40 (s, 1H), 7.66 (d, 1H, *J* = 16.1 Hz), 7.03 (d, 1H, *J* = 7.6 Hz), 6.84 (d, 1H, *J* =7.6 Hz), 6.25 (d, 1H, *J* = 16.1 Hz), 6.02 (s, 2H). ^{13}C NMR δ 169.3, 149.5, 148.2, 145.0, 128.6, 124.3, 115.9, 108.4, 106.4, 101.4.

3-(3,4-Methylenedioxyphenyl)propanoic acid **8** was obtained from acid **7** as pale yellow crystals in 75% yield. m.p., 77-78°C (Lit[6]. 87-88°C) ^{1}H NMR δ 6.75-6.62 (m, 3H, aromatic), 5.92 (s, 2H), 2.87 (broad t, 2H, *J*=7.4Hz), 2.62 (m, 2H); ^{13}C NMR δ 179.1, 147.6, 146.0, 133.9, 121.1, 108.7, 108.3, 100.8, 35.9, 30.3.

5-(3,4-Methylenedioxyphenyl)pentanoic acid **10** was obtained from piperic acid **9** as a brown solid in 98% yield, m.p. 95-96°C (Lit[6]. 96°C) ^{1}H NMR δ 8.87 (1H, broad), 6.73-6.58 (m, 3H, aromatic), 5.90 (s, 2H) 2.54 (broad t, 2H, *J*= 7.0 Hz), 2.35 (broad t, 2H, *J* = 7.0 Hz), 1.63 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 179.9, 147.4, 145.5, 135.8, 121.0, 108.7, 108.0, 100.6, 35.1, 33.8, 30.9, 24.0

Antibacterial activity:

Several compounds showed inhibitory activity against *Staphylococcus aureus*. The inhibitory activity of the aromatic acids in *series 1* and *series 2*, measured for different volumes of the acid solutions (10,000 ppm) used in the bioassay are shown in Figure 1. Compound **7** was not soluble in ethyl acetate, hence the results are not comparable.

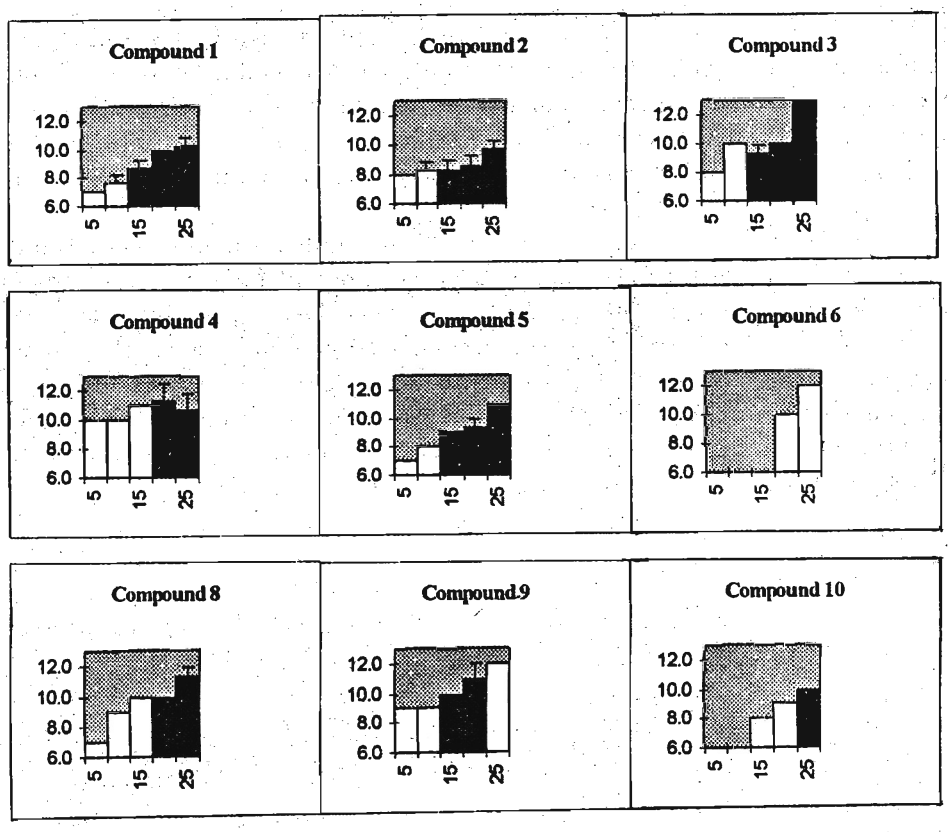


Figure 1: **Diameter of inhibitory zone in mm (y axis) against volume of test solution in** μl.
Bars indicate standard deviation. Dark columns and white columns indicate very clearly demarcated inhibitory zones and less clearly demarcated inhibitory zones respectively.

DISCUSSION

The spectroscopic data and comparisons with reported values confirm the formation of the required compounds in the Knoevenagel reaction, hydrogenation reactions and the $KMnO_4$ oxidation.

Only the very clearly demarcated zones were taken as inhibitory in the interpretation of bio-assay results. Comparison of the antibacterial activity of the acids in the first series with the corresponding acids in the second series shows that the activity is diminished by the presence of a 3,4-methylenedioxy substituent on the phenyl ring. This is to be expected if the mechanisms of action of the acids are similar to the action of sulfonamides which act as a folic acid synthesis inhibitor.[8] In contrast, comparison of activity of acid **4** and **9** shows a slight enhancement of activity in acid **9** at 15µl level. However this enhancement is not observed at greater than 15µl level.

Among the acids of the first series, hydrocinnamic acid **3** shows the highest antibacterial activity. Although hydrogenation of the double bond in compound **2** led to a marked enhancement of activity, similar hydrogenation of compound **4** had no significant effect on activity. In the second series, it was observed that hydrogenation of both double bonds in acid **9** decreased antibacterial activity.

Changes in antibacterial activity with the number of double bonds could be assessed by comparison of compound **2** with compound **4** in series 1. Compound **4** seems to be a better candidate only if we consider the less clear inhibition zones in the interpretation. An increase in number of carbons attached to the aromatic nucleus appears to decrease activity as seen by comparing the results for the pairs of compounds **3**/**5** and **8**/**10**. A plausible explanation for this behaviour is that the increase in hydrophobicity with increase in methylene groups affects the diffusion of these compounds on agar.

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